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Exploration of Lauric Acid as a Potentiator for Enhancing Warfarin Toxicity to Rats

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ABSTRACT

We conducted laboratory feeding trials to evaluate the effectiveness of lauric acid, a naturally occurring short-chain fatty acid, for enhancing the toxicity of warfarin to three species of Hawaiian rats (Rattus norvegicus, R. rattus, R. exulans). *We offered oats treated with one of three concentrations of (0.01, 0.1 or 1.0%) lauric acid and either of two concentrations (0.0125 or 0.005%) of warfarin to individually caged rats during a series of 24-h feeding trials. None of the warfarin–lauric acid combinations enhanced mortality in any of the three species. Nor did pre-exposing rats to lauric acid improve its effectiveness.* R. rattus *and* R. exulans *exposed to the highest concentration of lauric acid (1.0%) reduced their consumption of toxic baits. At the concentrations evaluated, lauric acid appears to have little potential as a bait additive for enhancing the toxicity of warfarin to rats.*

INTRODUCTION

R. exulans, *R. rattus* and *R. norvegicus* cause a variety of economic, health and ecological problems in Hawaii. They damage sugarcane (Pemberton, 1925; Doty, 1945; Hood *et al.*, 1970; Tobin *et al.*, 1990) and macadamia nuts (Fellows, 1982; Tobin, 1990); transmit zoonotic diseases such as bubonic plague (Dopmyer, 1936; Twigg, 1978; Tomich *et al.*, 1984) and leptospirosis (Tomich, 1979; Shimizu, 1984; Anderson & Minette, 1986);

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and prey on threatened and endangered plants (Stone, 1985), snails (Hadfield *et al.*, 1993), and birds (Kepler, 1967; Buxbaum, 1973; Atkinson, 1977).

Toxicants are the most practical means of managing rats over large areas (Moors *et al.*, 1992). Commercial rodenticides are easy to use, relatively inexpensive, and can be applied over large areas. Because rats reproduce throughout the year in Hawaii (Tamarin & Malecha, 1972; Tobin *et al.*, 1994), managers must apply control measures repeatedly or over prolonged periods. Most acute rodenticides rapidly lose their effectiveness with repeated use (Doty, 1945; Marsh, 1988; Sugihara *et al.*, 1995), and thus anticoagulant rodenticides may be more effective for long-term control.

Anticoagulant rodenticides have long been popular for commensal rodent control, and are increasingly being used throughout the world to control field rodents in both agricultural (Byers, 1981; Marsh, 1987; Bhat & Sujatha, 1989; Marsh, 1994) and conservation areas (Cruz & Cruz, 1987; Taylor & Thomas, 1989; Buckle & Fenn, 1992; Moors *et al.*, 1992). However, in the United States concerns about potential hazards to nontarget animals, particularly predators and scavengers, limit the number of registrations of anticoagulants for field use. In Hawaii, species of concern include the 'Io (Hawaiian hawk, *Buteo solitarius*), Pueo (Hawaiian subspecies of the short-eared owl, *Asio flammeus sandwichensis*), and 'Alala (Hawaiian crow, *Corvus tropicus*).

Enhancing the efficiency of anticoagulant baits would reduce the amount of bait needed to achieve control objectives and thus reduce possible environmental exposure. Past efforts to enhance the efficacy of anticoagulant rodenticides most often have been directed at reducing the cost of control programs and increasing the toxicity to resistant animals. Mechanisms evaluated included using bait additives that create a vitamin K deficiency (Krishnakumari & Muralidhara, 1977; Rao, 1979; Meehan, 1984), enhance the effects of hemorrhaging by affecting blood capillaries (Rao, 1979), or otherwise potentiate the effect of anticoagulants (Timm, 1994).

Several drugs (Brodie, 1965; Aggeler *et al.*, 1967; Solomon & Schrogie, 1967; Solomon *et al.*, 1968; O'Reilly, 1973; Welch, 1973; Pepper & Wosilait, 1977; Anonoymous, 1982) and free fatty acids (Solomon *et al.*, 1968; Chakrabarti *et al.*, 1976) potentiate anticoagulant activity in humans and other species by displacing anticoagulants from their binding sites on albumin. Such potentiation might provide a mechanism for enhancing the rodenticidal efficacy of anticoagulant rodenticides.

Lauric acid is a naturally occurring short-chain fatty acid that displaces warfarin *in vitro* from both human (Solomon *et al.*, 1968) and bovine (Chakrabarti *et al.*, 1976) albumin. Much of the warfarin ingested by rats binds to plasma proteins (Yacobi & Levy, 1975). Because lauric acid also

binds to plasma albumin, it might compete with warfarin for sites on the plasma albumin, thereby making more free warfarin available to exert its toxicological effects. This would decrease the amount of warfarin needed to achieve a desired level of control, thereby reducing potential environmental hazards. Thus, we conducted laboratory feeding trials to explore the potential of lauric acid as a bait additive for enhancing the toxicity of warfarin to three species of Hawaiian rats.

MATERIALS AND METHODS

Experimental animals

We captured *R. norvegicus*, *R. rattus* and *R. exulans* in and around sugarcane fields and forested areas near Hilo, HI and quarantined them at the Denver Wildlife Research Center's Hawaii Field Station for $\geqslant 14$ days before testing. We housed rats in individual stainless steel cages ($18 \times 18 \times 36$ cm) with *ad libitum* access to Rodent Laboratory Chow 5001® (Purina Mills Inc.) and water at 25°C on a 12 h light:dark cycle (reference to commercial products for identification does not imply endorsement by the authors or the U.S. Department of Agriculture).

We randomly assigned five males and five females of each species by weight classes to each of five treatment groups during experiment one and each of three treatment groups during experiment two. We used only healthy *R. norvegicus* and *R. rattus* weighing $\geqslant 90$ g and *R. exulans* weighing $\geqslant 35$ g.

Bait preparation

For each bait, we dissolved the appropriate amount of warfarin and/or lauric acid in a 1:1 mixture of acetone:methanol, added the resulting solution to slightly crimped rolled oat groats in four parts, and mixed it until the acetone–methanol solvent evaporated. We added Alcolec S® (lecithin; American Lecithin Company Inc.) at 2% (w/w) as an adhesive and protective overcoat and mixed until the oats no longer stuck together. In both experiments, we used lower concentrations of warfarin than that registered for use in commensal rodenticides to accentuate differences in mortality among treatments. We prepared a nontoxic reference bait (0.0% warfarin and 0.0% lauric acid) by overcoating untreated oat groats with Alcolec S at 2%. We air-dried all baits and stored them in a refrigerator at 5°C.

Feeding trials

During experiment one, we evaluated the efficacy of four warfarin (0.0125%)–lauric acid (0.00, 0.01, 0.10 or 1.00%) combinations and a

nontoxic reference. This experiment consisted of five consecutive 24-h feeding trials.

We conducted experiment two in two phases, each consisting of five consecutive 24-h feeding trials, to evaluate whether pre-exposing rats to lauric acid improved the effectiveness of a warfarin–lauric acid formulation. We offered rats oat groats treated with 0.0 (groups one and two) or 0.1% (group three) lauric acid during the pre-exposure phase, then offered oat groats treated with the 0.005% warfarin and either 0.0 (group one) or 0.1% (groups two and three) lauric acid during the toxic exposure phase.

In both experiments, we offered *R. norvegicus* and *R. rattus* 20 g and *R. exulans* 10 g of their assigned bait/animal/trial. Rodent laboratory chow and water were available *ad libitum*. We calculated daily bait consumption by adjusting the amount of bait offered for moisture gain or loss (based on changes in the weight of three samples of each bait that were exposed in the test room throughout each trial) and subtracting the weight of uneaten and spilled food. We did not monitor consumption of rodent chow. We observed rats for toxicosis or mortality during the feeding trials and for 14 days post-treatment.

Data analysis

We used SAS Univariate Procedures (SAS Institute Inc., 1988a) to test for normality and SAS GLM (General Linear Models) Procedures (SAS Institute Inc., 1988b) to perform nonparametric ANOVA on the ranked bait consumption and warfarin ingestion data. We used Duncan's Multiple Range Test (Saville, 1990) with an experimentwise-error rate of 0.05 to make pairwise comparisons among means. We analyzed consumption of warfarin-treated bait for only the first two trials of each experiment, thereafter toxicosis could confound the effects of lauric acid. We analyzed consumption separately for the pre-exposure (all five trials) and toxic exposure (first two trials) phases of experiment two. We characterized mortality using descriptive statistics.

RESULTS

Rattus norvegicus

Experiment 1

During the first two trials, consumption was similar among the five treatment groups ($F = 1.44$; d.f. = 4,90; $p = 0.23$) and between trials ($F = 0.00$; d.f. = 1,90; $p = 1.00$). Consumption appeared to decline

slightly during the last two trials for rats in the warfarin alone and warfarin +1.0% lauric acid groups (Fig. 1). Average total ingestion of toxicant per kg body weight and mortality were similar among the four groups exposed to warfarin, whether or not lauric acid was present (Table 1). The lone mortality in the 0% warfarin group probably died of natural

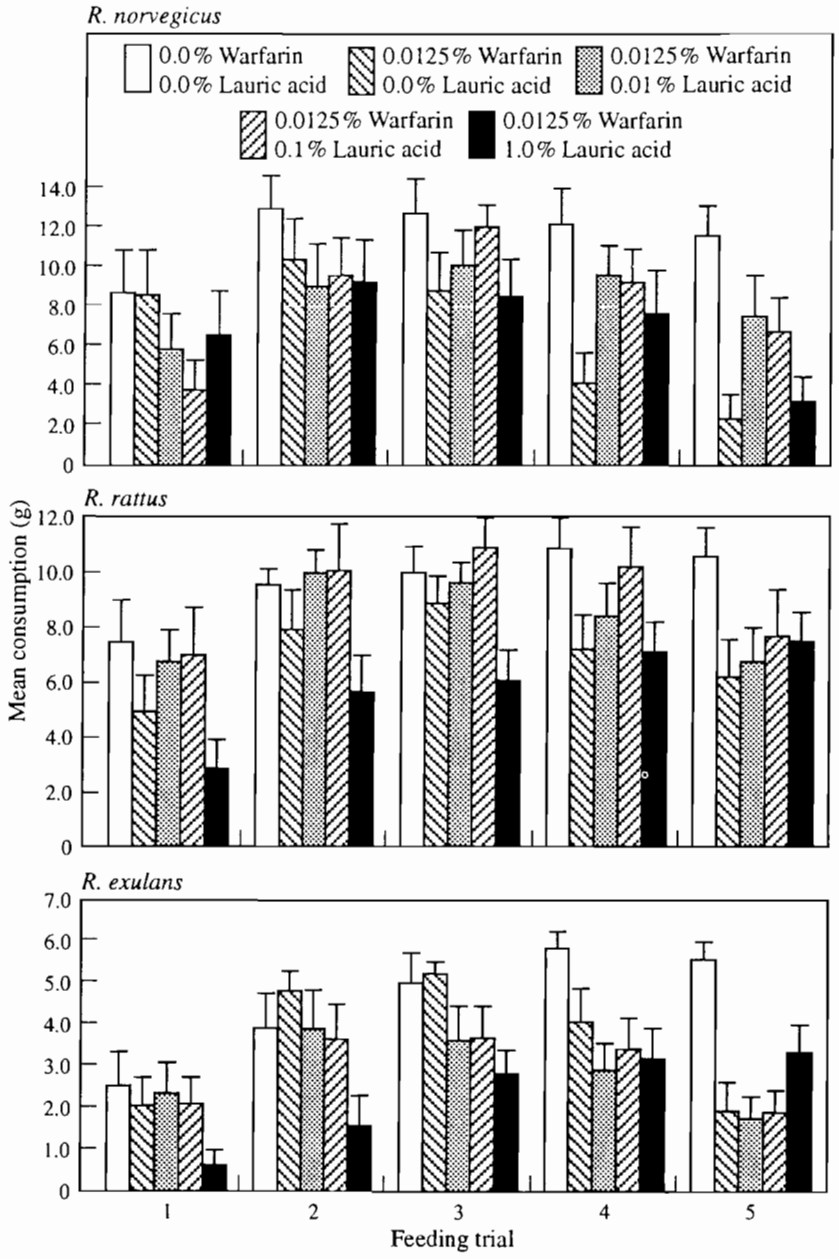

Fig. 1. Mean bait consumption (± SE) by rats (*Rattus* spp.) (10/group/species) offered oat groats treated with warfarin and lauric acid. Means include only animals that were alive when the baits for that trial were offered. Rodent laboratory chow and water were available *ad libitum* throughout the study.

TABLE 1

Mean mg/kg of Warfarin Ingested and Mortality of Rats Offered Oat Groats Treated with One of Five Combinations of Warfarin and Lauric Acid during Five 24-h Trials. Rodent Laboratory Chow and Water were Available *Ad Libitum* Throughout the Study

Species	Treatment		Mean mg/kg warfarin ingested (± SE)	Mean days to death (range)	No. dead/no. tested
	% Warfarin	% Lauric acid			
Rattus norvegicus	0.0000	0.00	0.0	14.0	1/10
	0.0125	0.00	22.2 (4.1)	5.7 (4–8)	7/10
	0.0125	0.01	27.3 (4.3)	9.4 (8–15)	7/10
	0.0125	0.10	25.4 (2.0)	7.2 (5–10)	9/10
	0.0125	1.00	22.4 (4.2)	5.9 (4–8)	7/10
Rattus rattus	0.0000	0.00	0.0	0.0	0/10
	0.0125	0.00	26.0 (2.4)	7.0 (5–9)	4/10
	0.0125	0.01	32.2 (2.9)	7.5 (4–10)	8/10
	0.0125	0.10	35.8 (5.0)	7.0 (4–9)	7/10
	0.0125	1.00	23.6 (4.1)	6.0 (4–8)	3/10
Rattus exulans	0.0000	0.00	0.0	0.0	0/10
	0.0125	0.00	32.6 (3.8)	7.0 (4–10)	10/10
	0.0125	0.01	26.1 (4.8)	7.3 (4–11)	4/10
	0.0125	0.10	26.1 (4.7)	6.4 (5–8)	8/10
	0.0125	1.00	21.0 (4.2)	5.7 (4–8)	3/10

causes. This animal appeared healthy and maintained its weight throughout quarantine and the experiment, but it did not eat any bait and died on day 14.

Experiment 2

Consumption was similar among the three groups during both pre-exposure ($F = 1.30$; d.f. = 2,135; $p = 0.28$) and toxic exposure ($F = 1.90$; d.f. = 2,54; $p = 0.16$). Consumption varied little among the five trials of the pre-exposure phase ($F = 0.00$; d.f. = 4,135; $p = 1.00$) or between the first two trials of the toxic exposure phase ($F = 0.00$; d.f. = 1,54; $p = 1.00$), although it appeared to decline during days 3–5 of the latter (Fig. 2). Both mortality and total ingestion of warfarin per kg body weight ($F = 1.90$; d.f. = 2,27; $p = 0.17$) were similar among groups (Table 2).

Rattus rattus

Experiment 1

Consumption during trials one and two varied among baits ($F = 3.86$; d.f. = 4,90; $p = 0.01$), but not between trials ($F = 0.00$; d.f. = 1,90; $p = 1.00$). Rats offered oats treated with the two lowest levels of lauric acid (0.01, 0.1%) consumed more bait than those offered the highest rate (1.0%), but not more than those offered warfarin alone (Fig. 1). Similarly, rats in the 0.01 and 0.1% lauric acid groups ingested more warfarin per kg body weight than those in the 1.0% group, but not more than rats in the warfarin alone group ($F = 14.59$; d.f. = 4,45; $p = 0.00$) (Table 1). Mortality mirrored consumption and was greatest in the 0.01 and 0.1% lauric acid groups (Table 1). Consumption, ingestion of warfarin per kg body weight, and mortality were similar for rats offered oats treated with 1.0% lauric acid and those offered oats treated with warfarin alone.

Experiment 2

Consumption varied among groups during both phases of the experiment (pre-exposure: $F = 4.60$; d.f. = 2,135; $p = 0.01$; toxic exposure: $F = 4.07$; d.f. = 2,54; $p = 0.02$). Rats in group two (0.0% lauric acid pre-exposure, warfarin + 0.1% lauric acid toxic exposure) consumed more during each phase than did rats in group one (0.0% lauric acid pre-exposure, warfarin alone toxic exposure) (Fig. 2). Consumption did not differ among feeding trials during either phase (pre-exposure: $F = 0.00$; d.f. = 4,135; $p = 1.00$; toxic-exposure: $F = 0.00$; d.f. = 1,54; $p = 1.00$). Total ingestion of warfarin per kg body weight varied little among groups of *R. rattus* ($F = 0.67$; d.f. = 2,27; $p = 0.52$) and was similar to the amount consumed

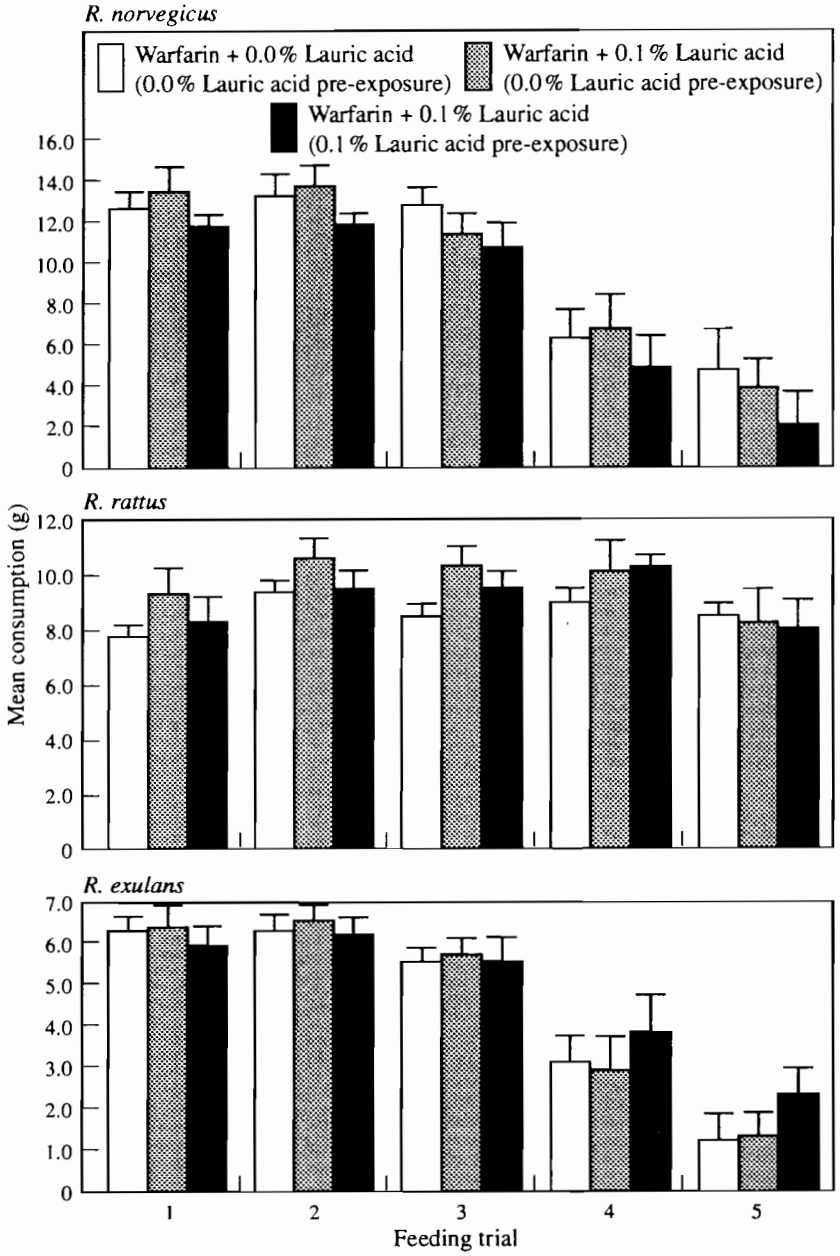

Fig. 2. Mean consumption (± SE) of oat groats treated with 0.005% warfarin and lauric acid by rats (*Rattus* spp.) (10/group/species) pre-exposed to oats treated with 0.0 or 0.1% lauric acid. Means include only animals that were alive when the baits for that trial were offered. Rodent laboratory chow and water were available *ad libitum* throughout the study.

TABLE 2

Mean mg/kg of Warfarin Ingested and Mortality of Rats Offered Oat Groats Treated with One of Two Concentrations of Lauric Acid during Five 24-h Pre-exposure Trials and Oat Groats Treated with Warfarin and One of Two Concentrations of Lauric Acid during Five 24-h Toxic Exposure Trials. Rodent Laboratory Chow and Water were Available *Ad Libitum* Throughout the Study

Species	Pre-exposure	Treatment		Mean mg/kg warfarin ingested (± SE)	Mean days to death (range)	No. dead/no. tested
	% Lauric acid	% Warfarin	% Lauric acid			
Rattus norvegicus	0.0	0.005	0.0	12.4 (0.9)	5.6 (4–8)	8/10
	0.0	0.005	0.1	12.5 (0.9)	4.9 (4–6)	9/10
	0.1	0.005	0.1	10.2 (0.8)	5.1 (3–7)	8/10
Rattus rattus	0.0	0.005	0.0	12.5 (1.0)	9.0 (8–10)	3/10
	0.0	0.005	0.1	13.8 (0.9)	6.6 (5–8)	5/10
	0.1	0.005	0.1	13.0 (0.7)	7.8 (5–9)	4/10
Rattus exulans	0.0	0.005	0.0	15.1 (1.5)	6.5 (6–8)	8/10
	0.0	0.005	0.1	15.0 (1.2)	5.9 (4–9)	7/10
	0.1	0.005	0.1	16.1 (1.8)	6.4 (4–12)	5/10

by *R. norvegicus*. With one exception, mortality was lower than for the other two species, but varied little among groups of *R. rattus* (Table 2).

Rattus exulans

Experiment 1

Consumption during the first two trials varied among baits ($F = 3.96$; d.f. = 4,90; $p = 0.01$), but not between feeding trials ($F = 0.00$; d.f. = 1,90; $p = 1.00$). Rats offered oats treated with warfarin + 1.0% lauric acid consumed less than did rats in any other group (Fig. 1). Consumption by rats offered 1.0% lauric acid appeared to increase during the last three trials, while consumption by rats in the other three warfarin groups declined, probably because of toxicosis. Rats offered oats treated with 1.0% lauric acid ingested less warfarin per kg body weight than did rats offered oats treated with warfarin alone ($F = 13.16$; d.f. = 4,45; $p = 0.00$) (Table 1). Of rats offered warfarin-treated oats, mortality was lowest in the highest lauric acid group (Table 1), and highest in the medium lauric acid and warfarin alone groups.

Experiment 2

Consumption varied among groups during the pre-exposure phase ($F = 3.25$; d.f. = 2,14; $p = 0.04$), but not during the toxic exposure phase ($F = 0.22$; d.f. = 2,54; $p = 0.80$). During the pre-exposure phase, the two groups offered 0.0% lauric acid-treated oats consumed differing amounts. Consumption was similar among the five trials of the pre-exposure phase ($F = 0.00$; d.f. = 4,14; $p = 1.00$) and between the first two trials of the toxic exposure phase ($F = 0.00$; d.f. = 1,54; $p = 1.00$), but appeared to decline during the later (unanalyzed) trials of the toxic exposure phase (Fig. 2). Although mortality varied among groups (Table 2), total ingestion of warfarin per kg body weight did not ($F = 0.09$; d.f. = 2,27; $p = 0.91$).

DISCUSSION

With few exceptions, lauric acid had little effect on either consumption or mortality. *R. exulans* and *R. rattus* consumed less bait treated with 1.0% lauric acid, indicating that at this concentration lauric acid is repellent to these species. The higher mortality of *R. rattus* offered 0.01 or 0.1% lauric acid during experiment one could have been due to either potentiation or increased ingestion of warfarin per kg body weight. The absence of enhanced mortality with 0.1% lauric acid during the second experiment, when ingestion of warfarin was comparable among groups (12.5–13.8 mg/

kg body weight), suggests that consumption was the more important factor. Pre-exposing rats to lauric acid did not enhance mortality of rats subsequently exposed to warfarin + 0.1% lauric acid.

Previous studies that demonstrated potentiation of anticoagulant activity by competitive displacement differed from ours in that they were *in vitro*, evaluated drug interactions using therapeutic doses and prolonged treatments, and/or involved different species (Brodie, 1965; Aggeler *et al.*, 1967; Solomon & Schrogie, 1967; Solomon *et al.*, 1968; O'Reilly, 1973; Welch, 1973; Chakrabarti *et al.*, 1976; Pepper & Wosilait, 1977; Anonymous, 1982). Our contrasting results might also reflect different definitions of 'increased anticoagulant activity'. Most therapeutic studies evaluate anticoagulant activity based on changes in prothrombin time. Most rodenticidal studies evaluate changes in mortality. We designed our study to detect increased mortality, not increased prothrombin time.

Increasing the concentration of free warfarin in the bloodstream might not necessarily enhance mortality. Rapid metabolism and excretion can mitigate toxic effects (Aggeler *et al.*, 1967; Gillette, 1973; Pepper & Wosilait, 1977). For example, phenylbutazone and tolbutamide initially increased prothrombin time of dogs by displacing dicoumarol from binding sites, but these drugs also induced liver microsomal enzymes that metabolized dicoumarol and ultimately reduced prothrombin times (Welch, 1973). Phenobarbital, chlordane and DDT similarly decreased warfarin's toxicity by stimulating its metabolism in rats (Ikeda *et al.*, 1968).

Potentiation of anticoagulant rodenticides probably depends on the particular anticoagulant–potentiator combination. The effectiveness of potentiators with the same mode of action may even vary. Administering tolbutamide to dogs on dicoumarol therapy produced greater elevation of prothrombin time, more spontaneous bleeding, and higher mortality than did phenylbutazone (Welch, 1973). Both ibuprofen and phenylbutazone induce gastric ulceration and bleeding and interference with wound healing, which may enhance their effectiveness as anticoagulant potentiators (Sridhara & Krishnamurthy, 1992).

We selected warfarin for study because of its widespread use and the large body of data available on its toxicological and physiological effects. However, potentiators might be more effective with second generation anticoagulant rodenticides. Both ibuprofen and phenylbutazone increased the toxicity of bromadiolone and brodifacoum baits to *R. rattus* and reduced time to death (Sridhara & Krishnamurthy, 1992). These second generation anticoagulants are more potent, have a longer plasma half-life, and are pharmacologically active longer than coumarin-type first generation anticoagulants such as warfarin (Bachmann & Sullivan, 1983; Breck-

enridge *et al.*, 1985; Mount *et al.*, 1986; Nahas, 1987; Hadler & Buckle, 1992).

Based on our results, lauric acid does not potentiate the rodenticidal effects of warfarin in rats. Additional research would indicate whether other substances enhance the toxicity of anticoagulants to rats.

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